

PROJECT NUMBER: 6908  
PROJECT TITLE: Smoke Condensate Studies  
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PERIOD COVERED: October, 1988

#### I. TSNA PRECURSORS

- A. Objective: To determine the precursors of MS TSNA.
- B. Results: Work continues on preparation of a suitable form of a nicotine salt for use as a model of bound nicotine. Attempts to use a SPEX Freezer/Mill to grind nicotine bitartrate crystals to the particle size desirable for subsequent microencapsulation showed that this procedure produces too low a throughput to be practical. Two large mortars have been procured to enable adequate material to be ground using a more conventional approach.

A nicotine amino acid ester has been prepared as a possible nicotine release compound to act as a model for bound nicotine. The compound, *t*-butyl 2-(3-pyridinyl)-1-pyrrolidinylacetate, is an analogue of an ethyl ester prepared by G. Chan earlier.

Sugars have been added to burley (Bu) CEL to simulate the level of sugars present in oriental (Or) CEL, and an RL prepared by spraying this mixture on Bu base web (BW). Cigarettes made from this RL will be smoked for TSNA determination. This experiment was designed as a result of data reported previously where Or + Bu CEL's gave reduced TSNA relative to burley CEL alone when both were added to BuBW and corresponding cigarettes smoked. FTIR-EGA studies have also showed that ammonia evolutions from BuCEL/BuBW were eliminated when OrCEL was mixed with BuCEL before the RL was prepared. It is postulated that sugars in OrCEL react with soluble ammonia in BuCEL to form amino sugars, which act as scavengers for nitrosating agents that cause pyrosynthesis of MS smoke TSNA.

An experiment was carried out to determine conditions necessary to extract nicotine from uncured tobacco leaves in order to allow the tobacco to be cured after removal of nicotine. This activity is being carried out to determine whether removal of nicotine before curing will result in lowering of MS smoke NNK from the cured tobacco. Greenhouse burley leaves obtained from R. Bass were divided in half along the midvein, and 5-6 half leaves were extracted with 2 L hexane or 5% ethanol in hexane, while some were retained as a control. The extractions were carried out overnight. Each sample was extracted with water in a blender, and the alkaloids partitioned into methylene chloride at pH 10. Nicotine analysis (GC/MSD) showed that hexane removed 35% of the nicotine while 5% ethanol/hexane removed 52%. Ripe burley leaves were obtained from J. Smiley, University of Kentucky (via David Connors). Ten leaves were extracted with 3 L of 5% ethanol/hexane on a shaker for 3 days, followed by evaporation of residual solvent and air-curing of the leaves. Ten additional leaves were treated in the same manner, and the solvent changed, followed by

an additional overnight extraction before curing. The control (10 leaves) was also subjected to air-curing.

- C. Plans: When correctly sized nicotine bitartrate particles are obtained, submit samples for microencapsulation, and add to appropriate fillers. Add the nicotine amino acid ester to filler. Determine the effects of these additions on MS TSNA deliveries. Smoke the BuCEL + sugar/BuBW cigarettes and determine TSNA for comparison to Bu + Or CEL/BuBW results. After the burley samples are air-cured for 7 weeks or more, they will be stemmed, cut, cigarettes prepared, and both filler and MS smoke analyzed for TSNA. The filler will also be analyzed for nicotine.

D. References:

Haut, S. A. Notebook 8595, p. 164.  
Morgan, W. R. Notebook 8579, p. 70.  
Warfield, A. H. Notebook 8558, pp. 193-195.

## II. ANALYTICAL PROCEDURES

- A. Objective: To develop and maintain analytical methodology for TSNA or other compound classes where information is needed for determining relationships of TSNA to their precursors.
- B. Results: Method development for simultaneous VNA/TSNA determination on the same smoke sample was continued. Addition of the VNA fraction to the TSNA fraction caused rapid deterioration of the chromatographic system. Addition of a precut to the alumina cleanup step failed to remove the cause of this problem. Further modifications to the method are being investigated.

Efforts were made to establish a GC/MSD method for nicotine and minor alkaloids. However, the approach utilized did not result in consistently reproducible response factors for these compounds. Therefore, a previously reported GC/NPD method using KOH/methanol extracts is now being reestablished. In this method, the internal standard and all components measured have the same response factor.

- C. Plans: Continue development of the VNA/TSNA method. Finish the reestablishment of the GC/NPD method for alkaloids and use it to analyze Project ART samples.

D. References:

Morgan, W. R. Notebook 8579, p. 70.  
Tickle, M. H. Notebook 8716, pp. 28-29.  
Levins, R. J. Notebook 8672, p. 80.

III. SUPPORT FUNCTION: CONDENSATE PREPARATION

- A. Objective: To fabricate cigarettes, perform smokings, and prepare condensate as needed for biological and chemical analysis.
- B. Results: Eleven samples prepared as part of the Crossed Solubles Base Web Study were sprayed on the specified fillers, and cigarettes made and smoked for S/M testing, along with the appropriate controls. Another RL sample based on BuBW (see I. above) was prepared from BuCEL + sucrose, fructose and glucose. Two other special cigarette samples were prepared and smoked for S/M testing.
- C. References:
- Hellams, R. D. Notebook 8613, p. 115.